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EDITORIAL

LIVESTOCK IMPROVEMENT

It has been said on many occasions that this country is the nursery from which the world may obtain its livestock. We have always been proud of the fact that the best stock in many parts of the world have been derived from ancestors bred in these islands, and that when new blood is required it is often from our own foundation stocks that the necessary animals are obtained. Evidence of these facts can be found in many parts of the world to-day, and, with the revival of world trade, in all probability further demands will be made on us for supplies of breeding livestock. It would appear that we have been fortunate in our breeding of the types of livestock which meet most of the requirements desired in animals to be kept and used in other parts of the world. Sometimes we wonder how this position has been reached. Inquiry will show that while no hard and fast rule has been adhered to, the quality of our livestock, which is found suitable for owners in other parts of the world, has been acquired through the experience and observations and intuition of some practical breeders, who have gradually built up in their studs, flocks and herds the desired type of stock. But, taking the country as a whole, we cannot feel satisfied or even proud of the quality of our livestock. There is ample evidence that sufficient attention has not been given, nor is it being given, to our breeding problems. The time has now arrived when we must give careful consideration to the subject and when fundamental research work must be undertaken. Attention must be devoted to the genetical make-up of the various types of livestock, and we must have the assistance of scientists trained in and familiar with genetics in order that a full study of the problem can be instituted. The type of study envisaged and required must, of necessity, be long-term, especially with the larger kinds of livestock; but no matter how long it takes, nothing but benefit to the breeders and the country will result. With poultry some progress has already been made, and it is clear that their genetic

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make-up can be so arranged that various desired qualities and characters can be forthcoming. The results of this type of work on some of the lower forms of animals which has engaged the attention of geneticists can probably be applied to livestock.

Steps have already been taken to improve some of the qualities and characters of livestock, e.g., the selection of bulls and boars for special characteristics. Such methods are all to the good and will undoubtedly yield results in the breeding of higher quality stocks. This, however, is not enough; we must institute research into the fundamental genetical make-up of the animal in order that the desired characters can be firmly and solidly established, and that the element of chance in breeding results will be largely eliminated. Science has now reached a stage when a considerable amount of knowledge is available on genetical methods; the time has arrived when they should be applied. Again, in any research into the subject we must keep in mind the objects we have in view. Not only must we work for the improvement or the stabilising of some specially desired character; we must take into account the purpose for which the animal is to be used. The object in view should be that animals may be so genetically bred that the desired and necessary qualities to suit all circumstances may be forthcoming.

In all this work we must not lose sight of the health factor —sound, fundamental health; for without a healthy animal our breeding efforts to improve livestock will matter but little. There are still many problems concerning the health of livestock to be studied, for even now we have to confess to much lack of knowledge on some of the essential body processes. Happily, we realise our ignorance of the subjects as well as their great importance, and we welcome the efforts already being made to improve our acquaintance with some of the essential parts of the subject. In the past we seem to have devoted our energies and time to the study of disease, rather than health. It is good that such studies should have been carried out, for from them it has been possible to devise and apply methods of control which have resulted, and will continue to result, in the prevention of much economic loss. We have still to continue such studies, but we must ply our energy also to a better understanding of basic health. This is especially important when we have to consider the many forms of animal husbandry in practice throughout the world and the different climatic conditions and environment to which livestock are submitted. The various scientists concerned have to work conjointly on these problems, and in particular the veterinary surgeon, because of his training and experience, has a major part to play in the evolution of the desired types of animals for the various purposes. Who knows—we may yet be able to evolve the fundamentally healthy animal with a resistance to many of the troublesome diseases which give rise to so much economic loss throughout the world.

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General Articles

CRITICAL ANTHELMINTIC TESTS IN CATTLE

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(1) Introduction

CONSIDERABLE doubt still exists concerning the most suitable drug for use in the treatment of intestinal nematodes of cattle in the tropics. Indeed, it is surprisingly difficult to sift out from the copious literature on anthelmintic treatment reliable information upon the efficiency of drugs against any particular helminth parasite of cattle. Most of the available information is based upon evidence such as the appearance of voided worms in the fæces, clinical improvement of the host, and a cessation or inhibition of egg-laying by the female worms, following the treatment. In the words of Dr. M. C. Hall (1926): "These data were inadequate for accurate judgment, clinical improvement could not be accurately measured, and the worms passed told nothing in regard to the worms remaining." He also pointed out the inadequacy of egg counts as an indication of the number of worms remaining in the animal, and it is now well established that the egg-laying rate of female nematodes varies with many factors, such as diet, total worm population, and the resistance of the host. It has been further demonstrated that various anthelmintics, such as chenopodium (Mhaskar, 1924) and phenothiazine (Gibson, 1945), exert an inhibitory effect on egg-laying which might be taken as evidence of anthelmintic efficiency, but that the egg count begins to rise again after an interval, which varies with the drug. It is evident, therefore that many of the drugs habitually used in cattle may prove worthless when their efficiency is assayed by a reliable test. Fortunately, the critical anthelmintic test adopted by Hall (1924) can be relied on to give accurate information of the anthelmintic efficiency of a drug, and is thus a valuable basis for its recommendation or condemnation. The test consists in the administration of a known dose of the anthelmintic drug to an animal of known weight by a uniform mode of administration, followed by collection and careful search for expelled worms in all the fæces passed by the animal over a certain number of days after treatment. The animal is then killed and the number of worms surviving in the alimentary canal is related to the number passed out in the fæces. Records of the application of this test to the treatment of cattle are few, probably owing to their size and expense. Some tests were carried out at the Veterinary Laboratory, Nigeria, in order that the best use might be made of the drugs available for the treatment of zebu cattle.

(2) Results

The following drugs were used in tests against the three most important intestinal nematodes of cattle in Nigeria, namely, *Hæmonchus contortus*, *Bunostomum phlebotomum* and *Œsophagostomum radiatum*.

Phenothiazine.
Gentian violet.
Copper sulphate and sodium arsenite.
Copper sulphate, sodium arsenite, and tobacco extract.

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Thymol.
Potassium antimony tartrate.
Brilliant green.
Tetrachlorethylene.
Carbon tetrachloride.
Oil of chenopodium.

The tests were carried out on ten healthy zebu yearlings, each weighing 120-130 kilograms. Each animal was isolated and drenched in the standing position with one of the drugs to be tested. In the case of carbon tetrachloride and tetrachlorethylene, the drug was immediately preceded by 300 c.cs. of 5 per cent. sodium bicarbonate (as recommended in Mönnig's text-book for use with tetrachlorethylene in cattle). The fæces passed by the animal were collected and thoroughly searched for expelled worms for six days after the drug was administered. The animal was then slaughtered, the abomasal and intestinal contents washed in a sieve (60 meshes per sq. inch), and the number of each of the three species of parasite remaining in the alimentary canal was counted. The efficiency of the drug against each parasite is expressed as the percentage of the total worm burden which was expelled. The results are shown in the table on facing page.

(3) Discussion

The results of these tests, inconclusive as they may be, owing to the fact that only one animal was used for each drug, show that none of the drugs at the dosage rate employed, was satisfactory in the zebu against *Bunostomum phlebotomum*, while only phenothiazine was of value in the expulsion of *Hæmonchus contortus* and *Œsophagostomum radiatum*.

A criticism that might be made is that stomach worms, having been killed by the anthelmintic, might be digested and thus fail to appear in the fæces, the efficiency of the drug being higher than it appeared. The results with phenothiazine, however, act as a check on this factor in that 64 *Hæmonchus contortus* were voided. Another criticism might be that the number of worms in the animal are, in some tests, small, but, as Hall (1924) pointed out, it is as difficult to secure 100 per cent. efficacy with a drug when only a few worms are present as when many are present. It now remains to examine these results and those of other workers to discover whether there are sufficient data available for stating which is the drug of choice in the treatment of the three bovine nematodes, *Hæmonchus contortus*, *Bunostomum phlebotomum* and *Œsophagostomum radiatum*.

Bunostomum phlebotomum. Hall (1923) seems to have performed the first critical tests with anthelmintics in cattle against this parasite, using carbon tetrachloride. He obtained 48 per cent. efficiency at a dosage rate of 0.88 c.c. per kilogram body-weight, and 99 per cent. at a dosage rate of 1.25 c.c. per kilogram. His dosage was much higher than that employed by the present writer, which was about 0.12 c.c. per kilogram, which probably explains the very poor result obtained. The animal used in the test by the present writer died six days after treatment, and the autopsy findings were suggestive of carbon tetrachloride poisoning, with fatty changes in the liver and heart. One of the animals treated by Hall died four days after treatment, and since that time there have been many reports on the toxicity of this drug for cattle (see Giese, 1932; Rose, 1932; and Lynch, 1932), and it seems that it is not safe for routine use in cattle.

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Table Illustrating the Effectivity of Ten Anthelmintic Treatments as Indicated by the Expulsion of Worms in the Fæces after such Treatment

Treatment	Parasite	Number of worms passed in the fæces 1st day	2nd day	3rd day	4th day	5th day	6th day	Number left in intestine	% Effect to nearest 0.5%
30 c.c.s. 5% copper sulphate 30 c.c.s. 1% sodium arsenite	Hæmonchus	0	0	0	0	0	0	220	0%
	Bunostomum	0	0	0	0	0	0	99	0%
	Œsophagostomum ...	0	6	0	0	0	0	over 300	less than 2%
30 c.c.s. 5% copper sulphate 30 c.c.s. 1% sodium arsenite 10 c.c.s. 6% tobacco extract	Hæmonchus	0	1	0	0	0	0	64	1.5%
	Bunostomum	1	0	0	0	0	0	43	2%
	Œsophagostomum ...	0	0	0	0	0	0	120	0%
½ oz. Tartar emetic in 8 oz. water	Hæmonchus	2	2	2	0	0	0	332	2%
	Bunostomum	0	0	0	0	0	0	277	0%
	Œsophagostomum ...	1	0	0	0	0	0	278	0.5%
200 c.c. 1% brilliant green	Hæmonchus	0	0	0	0	0	0	411	0%
	Bunostomum	0	0	0	0	0	0	4	0%
	Œsophagostomum ...	0	7	0	0	0	0	286	1%
100 c.c.s. 2% Gentian violet	Hæmonchus	0	0	0	0	0	0	520	0%
	Bunostomum	0	0	14	0	0	0	114	11%
	Œsophagostomum ...	0	0	0	0	0	0	110	0%
½ oz. Thymol in 2 ozs. glycerin	Hæmonchus	1	0	0	0	0	0	3,000	0%
	Bunostomum	0	0	0	0	0	0	5	0%
	Œsophagostomum ...	0	0	0	0	0	0	252	0%
120 minims of oil of chenopodium in 8 ozs. of linseed oil	Hæmonchus	2	0	0	0	0	0	605	less than 1%
	Bunostomum	0	0	0	1	0	0	280	less than 1%
	Œsophagostomum ...	2	0	0	0	0	0	223	less than 1%
5 c.c. carbon tetrachloride preceeded by 300 c.c.s. 5% sodium bicarbonate, repeated 3 times at intervals of 2 days	Hæmonchus	0	3	13	2	0	0	97	16%
	Bunostomum	1	1	0	2	0	0	395	1%
	Œsophagostomum ...	14	2	0	1	1	3 Died.	55	28%
20 c.c.s. tetrachlorethylene preceeded by 500 c.c.s. of 5% sodium bicarbonate	Hæmonchus	0	0	3	0	0	0	114	2.5%
	Bunostomum	0	0	0	0	0	0	68	0%
	Œsophagostomum ...	0	0	0	0	0	0	115	0%
30 gms. Phenothiazine in suspension in 8 ozs. water	Hæmonchus	0	62	2	0	0	0	0	100%
	Bunostomum	0	0	1	1	0	0	202	1%
	Œsophagostomum ...	0	64	10	1	0	0	8	90%

Swanson, Porter and Connelly (1940) carried out critical tests with phenothiazine on cattle. They found that unconditioned phenothiazine was of a high degree of efficiency against *Bunostomum phlebotomum* at a dose rate of 0.7-1.1 gms. per kilogram body-weight, but at a dosage rate 0.44-0.55 gms. it was much less effective, and Porter (1941), in a further series of tests, found that in doses of 0.1 gms. per kilogram body-weight it was not effective. The very poor result obtained by the present writer with this drug is probably explained by the low dosage rate employed, which was approximately 0.4 gms. per kilogram body-weight. Furthermore, the animal was not starved before treatment, as the tests were so designed as to be indicative of the effect produced in the "bush." It seems, therefore, that to be effective against this parasite phenothiazine must be used in high dosage. 100 gms. or more would have to be given to an animal weighing 130 kilograms. Taking into account the poor condition of the cattle in Nigeria at the time when treatment is required, such a dose may well reach the toxic level. This level seems to be lower in cattle than in sheep, according to Taylor and Sanderson (1940) and Roberts (1941): the latter urges that no animal under six months should be given over 30 gms. On the other hand, Lapage (1940) gave 85-125 gms., and Foggie (1940) gave 40-60 gms., to young calves, while Swanson *et al.* (1940) brought the dose rate up to 1.1 gms. per kilogram body-weight, all without ill effect. The matter as to whether phenothiazine is satisfactory against *Bunostomum phlebotomum* in doses which are non-toxic must therefore rest upon the results of Swanson, Porter and Connelly (1940), until further tests of its toxicity to cattle are carried out. If the drug is used in zebu cattle in Africa the dose must be about 100 gms. for a yearling. This would involve considerable expense. Cowsert and Berliner (1943) have recommended phenothiazine at a dosage rate of 20 gms. per 100 lb. body-weight. Other drugs which have been recommended against this parasite are thymol (Dawson, 1906), kamala and aloes (Duschanek, 1909, and Sprehn, 1925), copper sulphate (Caciedo Arboleda, 1939), turpentine (Reisinger, 1924), turpentine and creosote (Bedel, 1927), tetrachlorethylene (Mönnig, 1938, Poisson and Buck, 1938), and extracts of male fern (Distol) by Marek (1921), Sprehn (1925), Szigetwary (1931), and Kotlan and Mócsy (1941). Of these drugs, thymol, copper sulphate and tetrachlorethylene were found to be useless in the present writer's tests. The results with distol appear to be particularly promising, and critical tests with this drug, when it becomes available, would very probably prove it to be the drug of choice. According to Marek (1921), the dose for a yearling should be about 4.5 gms., repeated two or three times every 24 hours.

Hæmonchus contortus and Œsophagostomum radiatum. Hall's critical tests with carbon tetrachloride showed 100 per cent. efficiency against both these parasites, but, as already pointed out, the drug is too dangerous for use in cattle at such high doses. The critical tests by Swanson, Porter and Connelly (1940), and Porter (1941), demonstrated the high efficiency of phenothiazine against these two parasites of cattle. It was 100 per cent. effective at a dosage rate of 0.1 gms. per kilogram body-weight. Its efficiency is confirmed by the present writer, and it has been recommended for cattle in the treatment of hæmonchosis and œsophagostomasis by Roberts (1941) and Porter, Simms and Cauthen (1941). In the treatment of young cattle in West Africa, 50 gms. would be a satisfactory dose. In the present writer's tests, no other drug besides phenothiazine

was found to be effective against hæmonchus and œsophagostomum in cattle. Various other drugs have been recommended (see Hutyra and Marek, 1938), but there appears to be no basis of critical testing for this recommendation (see Hall, 1930).

(4) Summary

A series of critical tests were carried out on zebu cattle in Nigeria in order that the comparative efficiency of certain anthelmintic substances might be assayed. The results of these tests and a review of the literature on the subject led to the conclusion that phenothiazine is the most satisfactory drug yet known for the treatment of hæmonchosis and œsophagostomiasis in the zebu. A dose of 50 gms. is recommended for an animal weighing 120 kilograms.

The best treatment for bunostomiasis is still uncertain. Phenothiazine appears to be effective only at a dosage rate of 0.7-1.1 gms. per kilogram body-weight (Swanson, Porter and Connelly, 1940), but whether the drug would be toxic to the nomadic zebu calf in such high doses is open to debate. Various authors report very favourably on male fern extracts (Distol); a dose of 4.5 grams would appear to be satisfactory for young zebus. It should be repeated once or twice, if necessary, at 24-hour intervals (Marek, 1921).

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CHEMOTHERAPY OF ERYSIPELOTHRIX RHUSIOPATHIÆ INFECTIONS IN MICE

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FOLLOWING reports of *in vitro* antibacterial action by penicillin against *Erysipelothrix rhusiopathiæ*, together with evidence of some degree of protection in mice, there appears to have arisen a widespread impression that penicillin will prove of value in the treatment of this infection in swine. The evidence which has accumulated in these laboratories does not justify this inference, and a record of our own experiments with penicillin and some other chemotherapeutic agents may be of value.

Porter and Hale, in 1939[1], failed to protect mice infected with *E. rhusiopathiæ* with four-hourly sulphanilamide and sulphapyridine given intraperitoneally. The same treatment protected mice against strains of the allied organism, *Listerella*. Foley, Epstein and Lee, in 1944[2], found penicillin to be antibacterial *in vitro* to seven strains of *Listerella*. Heilman and Herrell, in 1944[3] [4], reported penicillin to be antibacterial *in vitro* to *E. rhusiopathiæ* and "exceedingly effective" in infections in mice. Libby and Holmberg, in 1945[5], reported penicillins G and X to be antibacterial *in vitro* to *E. rhusiopathiæ*. Konst, in 1945[6], treated infections of *E. rhusiopathiæ* in mice with sulphanilamide, sulphapyridine and sulphathiazole, and found all three to be ineffective. Hodgson, in 1945[7], reported the treatment of two cases of erysipeloid of the fingers and hand with penicillin intramuscularly. Rosler, in 1945[8], reports the failure of sulphathiazole and sulphapyridine to prevent the development of disease in deliberately infected swine. The prognosis of the infection in man is good.

Experimental

General.

Seven representative strains of *E. rhusiopathiæ* of varying pathogenicity to mice were examined. Soon after their isolation from known outbreaks of swine erysipelas they were freeze-dried in an effort to maintain their pathogenicity unchanged. They were 354 (Antwerp), 446 (Evans), 445 (S.E.*II*), and four strains received from Mr. Gledhill, of Cambridge, 1,061 (W.E.2), 904 (Venn), 905 (Runciman), and 353 (Gledhill).

In both *in vitro* and *in vivo* investigations, 18-hour cultures in nutrient broth, prepared from blood culture slopes of the dried organisms, were diluted to the concentration required. An inoculum of 0.1 ml. per 5 ml. of broth of a 1 in 1,000 dilution in broth of an 18-hour culture at 37.5° C. was used in the *in vitro* tests. Animal inoculation was by intraperitoneal injection of 0.5 ml. of a 1 in 10, 1 in 100, or 1 in 1,000 dilution of the culture in 5 per cent. mucin.

Dosage.

Penicillin, prepared by surface culture and recently assayed at 450 Oxford units per mgm., was dissolved in sterile glass distilled water and administered intraperitoneally. The sulphonamides were administered *per os* in the form of a suspension in 10 per cent. mucilage of gum acacia, or, if incorporated in the diet, a concentration of 1 per cent. in a modified Coward's diet[9].

Results

In Vitro.

Commencing at a drug concentration of 2,000 Oxford units of penicillin per litre of nutrient broth, or 2.0 grams per litre of nutrient broth in the case of the sulphonamides, dilutions in powers of 2 were made in 12 tubes, each containing 5 ml. of broth. An inoculum of 0.1 ml. of a 1 in 1,000 dilution of an 18-hour culture of *E. rhusiopathiæ* in nutrient broth was added to each tube and the results read after 18 hours' incubation at 37.5° C. The results (Table 1) show the concentration of penicillin in Oxford units, or grams of the sulphonamides, per litre which just inhibits the growth of the organisms.

TABLE 1.

Strain	Penicillin in units per litre	Sulphathiazole	Sulphanilylguanidine	Phthalylsulphathiazole	3-Sulphanilamidobenzamide	"Sulphetrone"	Benzylamine-4-Sulphonamide
		Grams per litre					
354	125	>2.0	>2.0	>2.0	1.0	>2.0	0.062
1,061	125	>2.0	>2.0	>2.0	1.0	>2.0	0.125
446	125	>2.0	>2.0	>2.0	0.5	>2.0	0.031
445	125	>2.0	>2.0	>2.0	1.0	>2.0	0.031
905	125	>2.0	2.0	>2.0	2.0	>2.0	0.062
904	125	>2.0	>2.0	>2.0	0.5	>2.0	0.062
353	125	>2.0	>2.0	>2.0	1.0	>2.0	0.062
Str. pyogenes (P 90)	7.5	0.0037	0.25	0.125	0.50	0.0037	0.031
S. aureus (133)	15.6	0.0037	0.50	0.015	1.0	0.031	0.031

The table shows the concentration of penicillin, a series of sulphonamide drugs and benzylamine-4-sulphonamide which just inhibits an inoculum of strains of *E. rhusiopathiæ* and strains of *Str. pyogenes* and *S. aureus*. *E. rhusiopathiæ* is seen to be highly resistant to sulphonamides, somewhat resistant to penicillin and quite susceptible to benzylamine-4-sulphonamide.

All the strains of *E. rhusiopathiæ* are highly resistant to those sulphonamide drugs which owe their mode of action to the reversal of *p*-aminobenzoic acid, and susceptible to benzylamine-4-sulphonamide and to penicillin, both of which act by a different antibacterial mechanism. It may be deduced that *E. rhusiopathiæ* does not utilise *p*-aminobenzoic acid as a growth factor. The usual range of activity obtained with these antibiotics, under the conditions of these experiments, is shown with *Str. pyogenes* (P 90) and *S. aureus* (133).

Action of Sulphonamides—In Vivo.

The range of sulphonamide drugs, incorporated in the modified Coward's diet, so as to give a concentration of 1 per cent. of drug, was offered to groups of 10 mice, one day prior to and during the 3 days following infection of the mice. Each mouse was infected with 0.5 ml. of a 1 in 10 dilution in 5 per cent. mucin of an 18-hour culture of *E. rhusiopathiæ* (354). A control group was injected with mucin only. The results (Table 2) show that sulphonamides in the diet give little protection.

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TABLE 2.

Treatment. All drugs 1 per cent. in the diet.	1 day.	2 days.	3 days.
Sulphanilylguanidine	10/10	3/10	0/10
3-Sulphanilamidobenzamide ...	10/10	3/10	0/10
Phthalylsulphathiazole ...	10/10	4/10	0/10
Controls	10/10	4/10	0/10
Mucin controls	10/10	10/10	10/10

The table shows the number of survivors from groups of 10 mice, infected with *E. rhusiopathiæ*, on diets containing 1 per cent. of sulphonamide drugs.

Since a loss of appetite following the infection may have contributed to the lack of therapeutic effect, a second experiment, in which the drugs were administered *per os*, was planned. The dose, given as a suspension in 10 per cent. mucilage of gum acacia, was 20 mgm. per mouse, given immediately and 6 hours following infection, and thereafter morning and evening until the controls were dead.

The results (Table 3) show that when force-feeding is used the infected animals survive a little longer, but the difference appears to be without practical application. The two series of results, when taken together with the *in vitro* results, justify the conclusion that the range of sulphonamides examined is ineffective in the chemotherapy of *E. rhusiopathiæ*.

Benzylamine-4-sulphonamide, which has no systemic activity (10, 11, 12, 13), was not included.

TABLE 3

Treatment, *per os*.	1 day.	2 days.	3 days.	4 days.
Sulphanilylguanidine	9/10	8/10	4/10	1/10
3-Sulphanilamidobenzamide ...	10/10	4/10	4/10	3/10
Phthalylsulphathiazole	8/10	7/10	4/10	2/10
Controls	6/10	4/10	0/10	
Mucin controls	10/10	10/10	10/10	10/10

The table shows the number of survivors from groups of 10 mice infected with *E. rhusiopathiæ* treated with sulphonamides *per os*.

Action of Penicillin—In Vivo.

The antibacterial activity of penicillin against *E. rhusiopathiæ* showed inhibition of growth, but at somewhat higher concentrations than that obtained against the usual range of penicillin-susceptible organisms. In planning a chemotherapeutic comparison large doses of penicillin were therefore used. Groups of 10 mice were infected with the strains of *E. rhusiopathiæ*, using 1 in 10 and 1 in 1,000 dilutions of the 18-hour cultures in 5 per cent. mucin, and penicillin in doses of 10 and 100 Oxford units, in 0.5 ml. of sterile glass distilled water, per mouse, was administered intraperitoneally morning and evening until all the controls were dead.

The results (Table 4) show that even the larger dose of 100 Oxford units of penicillin per mouse, given on six occasions, gives only temporary protection. The

course of the infection in the groups of mice treated with 10 Oxford units of penicillin did not differ from the untreated control group. A slight degree of protection is seen with large doses of penicillin against all seven strains of *E. rhusiopathiæ*. This low degree of protection obtained with penicillin is less than that expected from the results of *in vitro* tests, particularly in view of the observed relationship between the results of *in vitro* and *in vivo* tests with the Gram-positive pathogens.

TABLE 4.

E. rhusiopathiæ.	Dilution of the organism in 5% mucin.	Treatment.	Number of days. 1	2	3	4	5
1,061	1/10	Penicillin (100 O.U.) ...	7	7	4	2	1
		Controls	8	1	0		
	1/1,000	Penicillin (100 O.U.) ...	10	8	6	4	0
		Controls	10	4	0		
446	1/10	Penicillin (100 O.U.) ...	10	7	4	1	
		Controls	9	3	1	0	
	1/1,000	Penicillin (100 O.U.) ...	9	8	4	4	2
		Controls	10	6	1	0	
445	1/10	Penicillin (100 O.U.) ...	10	9	6	5	2
		Controls	10	0			
	1/1,000	Penicillin (100 O.U.) ...	10	10	9	7	5
		Controls	10	8	1	0	
905	1/10	Penicillin (100 O.U.) ...	10	5	3	0	
		Controls	8	1	0		
	1/1,000	Penicillin (100 O.U.) ...	10	6	4	2	0
		Controls	10	2	0		
904	1/10	Penicillin (100 O.U.) ...	9	2	0		
		Controls	9	0			
	1/1,000	Penicillin (100 O.U.) ...	10	6	3	1	0
		Controls	10	4	0		
353	1/10	Penicillin (100 O.U.) ...	8	1	0		
		Controls	6	0			
	1/1,000	Penicillin (100 O.U.) ...	8	5	2	1	0
		Controls	3	0			
354	1/10	Penicillin (100 O.U.) ...	10	10	5	1	0
		Controls	10	0			
	1/1,000	Penicillin (100 O.U.) ...	10	10	8	2	0
		Controls	10	5	1	0	

The table shows the number of survivors from groups of 10 mice, infected with *E. rhusiopathiæ*, treated with penicillin intraperitoneally.

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Discussion

Following the report of the effective antibacterial action of penicillin (3, 4, 5), and of the effective chemotherapy with penicillin in mice (3, 4), against *E. rhusiopathiæ*, an impression has arisen that effective chemotherapy in swine may be assumed. Our experiments do not support this view. It has been shown that sulphathiazole, sulphanilylguanidine, phthalylsulphathiazole, sulphetrone (an example of a sulphone type of drug), and 3-sulphanilamidobenzamide possess no antibacterial activity and no chemotherapuetic activity against strains of *E. rhusiopathiæ*. This confirms the observations of Porter and Hale[1], and Konst[6]. The sensitivity of *E. rhusiopathiæ* to benzylamine-4-sulphonamide and penicillin, together with its resistance to the antibacterial and chemotherapeutic activity of the range of sulphonamide drugs used, justifies the deduction that *E. rhusiopathiæ* does not use *p*-aminobenzoic acid as a growth factor.

On the other hand, the degree of antibacterial activity shown by penicillin against *E. rhusiopathiæ in vitro* justified the expectation of successful chemotherapy. Heilman and Herrell[3] found that three strains of *E. rhusiopathiæ* were completely inhibited by 0.10 Oxford units of penicillin per ml., and Libby and Holmberg[5], using penicillins G and X, found penicillin G to be inhibitory at 0.05 Oxford units per ml., and penicillin X to be inhibitory at 0.16 Oxford units per ml. The strains of *E. rhusiopathiæ* examined here are inhibited at 0.12 Oxford units of penicillin per ml. In our experience with penicillin-susceptible organisms this degree of bacteriostasis *in vitro* should not require the large doses of penicillin *in vivo* which are necessary to show even a temporary protection in mice. We can offer no explanation for this anomaly.

Heilman and Herrell[3], using doses of 1,000 Oxford units of penicillin per mouse per day achieved a 95 per cent. protection in infections due to *E. rhusiopathiæ*. These must be labelled " enormous " doses. W. E. Herrell[4] has inferred that penicillin might prove exceedingly effective *in vivo* against infections in swine due to *E. rhusiopathiæ*. The expermental evidence reported here and elsewhere should lead to a more cautious view. Should swine erysipelas prove amenable to penicillin therapy, the dosage necessary is likely to be very large and the course of the disease protracted.

Summary

Seven strains of *Erysipelothrix rhusiopathiæ* were fast to sulphathiazole, sulphanilylguanidine, phthalylsulphathiazole, 3-sulphanilamidobenzamide and sulphetrone, and did not prove amenable to chemotherapy with these drugs.

E. rhusiopathiæ is sensitive to the action of penicillin and benzylamine-4-sulphonamide *in vitro*.

Penicillin, even in high doses, exerts little protective action in mice infected with *E. rhusiopathiæ*.

The results in mice do not justify the conclusion that infections of *E. rhusiopathiæ* in swine will prove readily amenable to chemotherapy with penicillin.

The author desires to acknowledge the help and criticism of Dr. G. Brownlee in the preparation of the paper, and the technical assistance of Mr. M. W. Cheeseman in the investigations.

Near Pastern Joint.

Off Pastern Joint.

[*See Page 93*

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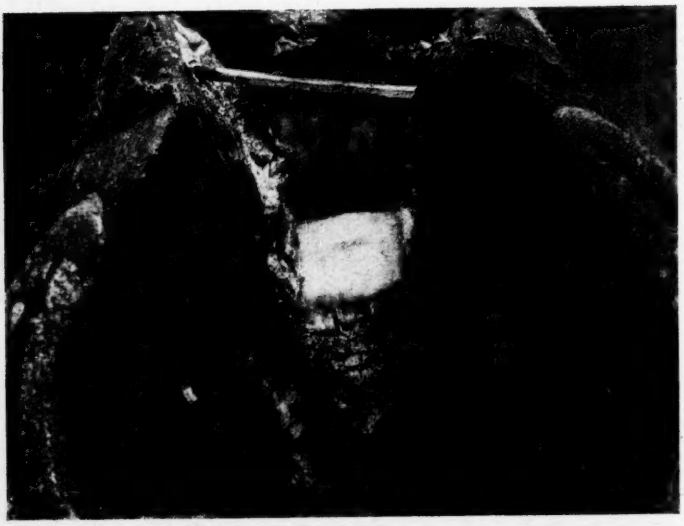
Near Navicular bone exposed.



Off Navicular bone exposed.

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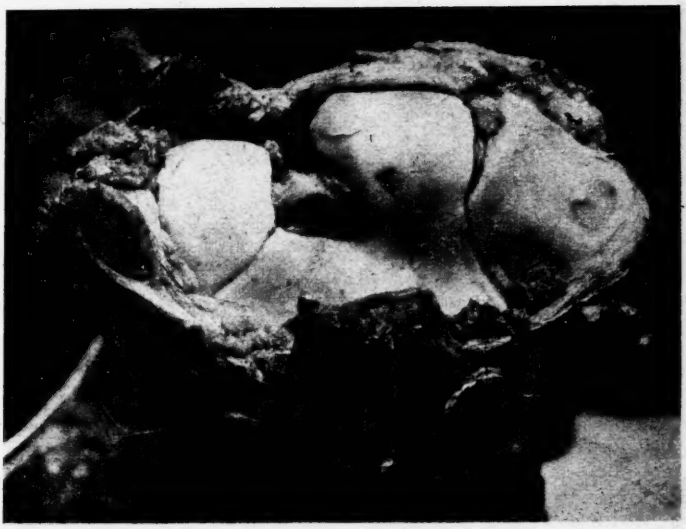

Near Carpus.

[See Page 93

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Off Carpus.

See Page 93]

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A CASE OF MULTIPLE ARTHRITIS IN A THOROUGHBRED

By V. BERWYN JONES, M.R.C.V.S.,

Wonersh.

THE subject was a three-year-old thoroughbred entire; a big slashing horse, but flat sided and apple jointed. He had never been "backed" because of his overgrowth. Exercise had all been in hand. He was not a free mover, although he went incredibly well in view of the P.M. findings. In spite of treatment the joints continued to enlarge and as it was considered that he was unlikely to stand training he was destroyed. The P.M. revealed what appeared to be a state of ulceration in the articular cartilages. The illustrations depict those found in the carpus and distal joints.

At each site the articular cartilage was so eroded as to be perforated, and this perforation connected with an underlying bone caries. This carious area was connected by a rather narrow strip to the cartilage and at the distal end of the lesion it was expanded to about the size of a threepenny piece.

It is remarkable how well the horse went considering the multiplicity and extent of the lesions. As the horse had never been in work it is probable that the initial cause was associated with faulty nutrition rather than with trauma.

Prof. McCunn informs me that he has frequently observed similar lesions in elbow, stifle and tibio-astragaloid joints of dissecting room and slaughterhouse specimens which had shown no evidence of lameness in life. On another occasion in which the stifle was affected there was an almost complete absence of the inter-articular semi-lunar fibro-cartilage. This latter animal moved quite well before destruction.

Whatever the cause of the erosion or ulceration the condition would appear to be to all intents a painless one which is remarkable considering the amount of pain generally evoked by even slight injuries to joints.

I would like to hear your readers' comments.

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INCIDENCE OF PATHOGENIC BACTERIA IN MARKET MILK OF CAIRO

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It has always been a matter of concern to the sanitary authorities to ensure a clean supply of milk for their localities, because serious epidemics have been frequently spread through milk and its products. In 1932 Wahby published statistics on milk epidemics compiled up to 1929 showing that in the U.S.A., England and other countries 1,465 milk epidemics involved 92,874 persons.

From the sanitary point of view it is not only important to produce milk free from disease-producing bacteria, but also it must be of low bacterial count and almost free of B. coli.

Therefore it is our purpose here to investigate the sanitary condition of market milk of Cairo City as regards (1) the bacterial count and B. coli. titre, and (2) the incidence of some important pathogenic organisms, i.e., mycobacterium tuberculosis, brucella abortus and the enteric fever group (eberthella typhosum and salmonella paratyphi).

Experimental

Two hundred milk samples were examined. They were taken from the shops, street salesmen and dairy byres in such order that they were evenly distributed among the different sections of Cairo. The samples were collected in sterilised glass bottles with glass stoppers; each sample consisted of half a pound of milk, and it was labelled to indicate the date, time, origin and the number of the sample. The samples were taken directly to the laboratory and examined, except very few samples which were kept in the ice-chest for not more than four hours. Preservatives were not used.

Each sample was first of all subjected to Storch's test to determine whether it was raw, boiled or mixed raw and boiled milk. To 5 c.c. of the milk was added two drops of H_2O_2 and two drops of a 2 per cent. solution of paraphenelynediamin. The mixture was shaken well, in raw milk the colour changed directly to indigo blue; mixed milk (raw and boiled), a grey blue colour developed immediately or in half a minute; boiled milk kept its white colour or gave a violet precipitate.

Before running the bacteriological examination each sample was diluted to 1 :100 and consequent dilutions up to 1 :1,000,000, these dilutions were only used for determination of the bacterial count and coli titre.

For the sake of convenience, the experimental technique may be better described in the following order :

Part I : (a) bacterial count (b) B. coli titre.

Part II : (a) mycobact. tuberculosis; (b) brucella abortus; (c) E. typhosum and salmonella paratyphi A and B.

Part I.

(a) Bacterial count :—Two methods were used : (1) The microscopic count; (2) The plate count.

(1) The microscopic or direct count:—After comparing the counts of both Breed's and Skar's methods on 10 milk samples Skar's method has been adopted throughout our work and its technique was as follows:

In a sterile test tube 0.2 c.c. of carbolic methylene blue solution was measured, to which were added 4.8 c.c. of the thoroughly mixed milk sample and 0.02 c.c. of 30 per cent. sodium hydroxide solution. The tube was shaken well and left to stand for a few minutes to allow the alkali to act. 0.02 c.c. of this mixture was evenly spread on a previously marked area of 4.8 sq. cm. on a clean slide and allowed to dry on a level surface. The film was then examined under a Leitz microscope which was standardised to give a field area of 0.031416 sq. cm. (tube length 16 cm., occular 6X, oil-immersion lens 1/12). The average number of bacteria per field was taken after counting 10 representative fields. The total number of bacteria per c.c. of milk was computed according to the following equation:

$$N = \frac{S\,F}{A\,a}$$

N = Number of bacteria per c.c. of milk.
F = Average number of bacteria per field.
a = Amount of milk in film in c.c.
S = Total area of film in sq. cm.
A = Area of microscopic field in sq. cm.

(2) The plate count: Both skimmed milk agar and lactose agar were tried on 10 samples and both were found to give almost identical results. However, lactose agar was more transparent and its colonies were easier to detect. Thus it has been preferred and used throughout the entire work. Four Petri dishes were prepared for each sample, two of which were inoculated each with 1 c.c. of the 1:10,000 milk dilution and the other two dishes each with 1 c.c. of the 1:100,000 dilution. The melted and cooled medium was poured into each plate aseptically and mixed thoroughly by a rotatory movement. The results were taken after 48 hour incubation at 37° C. Counting of the colonies was done with Wolfhügel counting apparatus. The obtained number of colonies from each plate was multiplied by the dilution used in that plate, and the result was recorded as the number of bacteria per c.c. of milk sample examined.

(b) B. coli titre: Three different media were preliminarily tried out on 10 milk samples, i.e., lactose broth, McConkey's bile salt lactose broth, and Kessler's and Swenarton's bile lactose peptone broth. The results obtained from Kessler's and Swenarton's excelled those obtained by the lactose broth and were identical with those obtained from McConkey's, but as Swenarton's was cheaper and easier to prepare than McConkey's, it has been used throughout the whole work.

The milk dilutions used were 1:1,000, 1:10,000, 1:100,000, and 1:1,000,000. From each dilution two tubes of medium were inoculated, each with 1 c.c. of the dilution. The first reading was taken after 24 hour incubation, and the second after 48 hour incubation. The highest dilution in which one or two tubes showed gas formation was recorded as B. coli titre of the sample.

Part II.

(a) Mycobacterium tuberculosis: Each milk sample was examined for the incidence of tubercle bacilli by the following three methods: (1) The microscopic method; (2) The cultural method; (3) The animal inoculation method.

(1) The microscopic method: The technique adopted was that recommended

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by Torrance and modified by Matthews, Maitland and Mary: 10 c.c. of the well-mixed sample were centrifuged at 2,500 r.p.m. for three minutes. The cream and supernatant fluid were poured off and two loopfuls from the sediment were spread evenly on a clean slide in a not too thin film. The smear was air dried, fixed and stained carbol fuchsin and decolourised with 3 per cent. HCl in 70 per cent. alcohol (Dounham). The film was first examined by the low power for the detection of any cell-grouping if present, and then by the oil immersion lens for the acid fast bacilli.

(2) The medium used was that formulated by Wahby in 1932; it was composed of yeast extract, peptone, glucose, potato flour, egg-yolk and glycerine. Brilliant green was added to prevent the germination of spore-formers. The final pH of this medium was 7.1—7.2. The milk sediment of centrifuged samples was treated after the method developed by Wahby in 1932. It consisted of mixing the sediment with 10 c.c. of normal solution of KOH for 10 minutes, then centrifuged for five minutes. The supernatant fluid was poured off and 10 c.c. of 7 per cent. HCl (2.45 actual percentage) were added to the sediment and thoroughly mixed for 10 minutes, after which it was centrifuged for five minutes. The supernatant fluid was poured off and the sediment was inoculated on two tubes which were incubated for eight weeks at 37° C. and inspected weekly. The tuberculous growth was confirmed by microscopical examination and typed by rabbit inoculation.

(3) The animal inoculation method: The untreated sediment and part of the cream from 40 c.c. milk were suspended in sterile saline solution and injected into a guinea-pig intramuscularly in the right thigh. The inoculated guinea-pigs were kept under observation for seven weeks, after which period they were killed and examined for tuberculous lesions. If tuberculous lesions were present, cultures were made from the emulsified and treated lesions, and another guinea-pig was inoculated with the infected material for confirmation. The isolated organisms were afterwards typed by inoculating mature rabbits subcutaneously with an emulsion of the pure culture. If the rabbit died within a few weeks after inoculation with generalised tuberculosis the organism would be considered bovine type, while if the animal survived this period and the lesions were localised in the lungs and kidneys the organisms would be typed human.

(b) Brucella abortus: Each sample was examined for the presence of B. abortus by the two following methods: (1) The agglutination test of the milk serum. (2) The animal inoculation.

(1) The agglutination test: 60 c.c. of the milk sample were centrifuged and 20 c.c. of the middle milk were transferred to a sterile test tube by a sterile pipette; a few drops of Rennet and three drops of 0.5 per cent. carbolic acid were added (Klimmer). The tube was then put in a water bath at 40° C. for half to one hour for coagulation, after which the milk serum was filtered through filter paper to make it clearer. One c.c. of the clear whey was added to a tube containing 4 c.c. of 0.5 per cent. carbolic saline solution, thus making a 1 :5 whey dilution. Six agglutination tubes, each with 0.5 c.c. of diluted antigen, were prepared. To the first agglutination tube 0.5 c.c. of the diluted whey was added to make a dilution of 1 :10, and then by means of normal saline a series of dilutions were made up to 1 :320. Two agglutination tubes were used as controls, one containing antigen and saline and the second containing saline and whey. All tubes were

incubated at 37° C. overnight, then removed from the incubator and left to cool for two hours at room temperature and the first reading was taken. When there was a doubt the rack was left for next morning at room temperature and a second reading was taken.

Before its use the antigen was diluted with normal saline to a density equal to one billion dead organisms per c.c.

(2) The animal inoculation test: In order to economise in the number of guinea-pigs used in this work, the inoculated guinea-pigs were examined at the same time for both tubercle bacilli and Br. abortus lesions (Pullinger, Wilson and Nutt). The blood serum of the guinea-pigs which were killed after seven weeks was collected in sterile flasks to be used for agglutination test in addition to the autopsy and the bacteriological examination of their internal organs. It was impossible to obtain blood serum from those guinea-pigs which died during the course of the experiment, therefore we had to be satisfied with the examination of their internal organs, especially the spleen, liver and regional lymphatic glands, for the presence of characteristic lesions. In all cases films were made from such organs and stained with Gram's stain. If Gram negative short bacteria were found in the organs, cultures from them were made on liver-agar medium with gentian violet 1 :200,000 and incubated under a 10 per cent. CO_2 tension (Huddleson) at 37° C. for four days. Films from suspected colonies were examined microscopically and subcultures were made.

For typing the isolated Brucella organism the following two methods were used:

(1) Making cultures on beef infusion agar with thionin 1 :30,000, as Br. abortus does not grow on such medium while Br. melitensis can grow. Tubes of beef infusion agar without thionin were inoculated as controls.

(2) A suspension of the isolated organisms was standardised to be used in the agglutination test as an antigen against a known rabbit immune serum. This method is universally considered the surest method for typing Br. abortus.

(c) The enteric fever group: At the beginning of this work direct inoculation of McConkey's medium with part of the cream and the sediment obtained from centrifuging 20 c.c. of each milk sample was tried.

The plates were incubated at 37° C. for 24 hours, after which the plates were examined for non-lactose fermenters. This method proved to be unreliable, on account of the tremendous numbers of bacteria found in the examined milk. Therefore we tried another method for isolation of the enteric fever organisms; 50 c.c. of milk were mixed with brilliant green to make a concentration of 1 :25,000, and incubated at 37° C. for about 12 hours. But the milk was found to be frequently coagulated before any isolation could be made; thus this method had to be abandoned.

A third method was tried and proved successful; it was as follows:

Three tubes of peptone water containing brilliant green in concentration of 1 :25,000, 1 :40,000, and 1 :70,000, successively, were inoculated with two loopfuls from each sediment and part of the cream of centrifuged 20 c.c. milk, and incubated at 37° C. for 12 hours, after which McConkey's plates were inoculated with two loopfuls from the different peptone tubes and incubated at 37° C. for 24 hours. Films from non-lactose-fermenting colonies were examined microscopically,

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and subcultures were made from the Gram-negative bacteria, first on McConkey's plates and then on ordinary agar medium.

Parallel experiments made on mixtures of coli and paratyphoid bacteria with both the brilliant green and tetrathionate (0.5 per cent.) enrichment methods proved that both methods gave almost identical results, and, therefore, the first was adopted, specially when it has been found that the typhoid organisms were rather sensitive to the tetrathionate (Knox, Gell and Pollack).

Suspicious organisms were identified as follows:

(1) Biochemical test: Tubes of 1 per cent. lactose, 1 per cent. glucose, and 1 per cent. mannite were inoculated and incubated at 37° C. to determine whether the organisms were E. typhosum or Salm. paratyphi.

(2) Agglutination test: It is made with suspension of the suspected organisms against known immune sera of E. typhosum and Salm. paratyphi, A or B.

(3) Culture: By inoculating tubes of litmus milk medium to determine whether the organisms were Salm. paratyphi A or Salm. paratyphi B.

The Results

Part I. (a) The bacterial count and B. coli titre: In this work Skar's method excelled the lactose agar plate method in giving higher counts. Skar, himself, stated that his method usually shows from 2 to 70 times as many bacteria as obtained by the plate method, due to the fact that many organisms may be dead and many others may clump together to form one colony.

The highest count obtained by Skar's was 332,108,006 bact. per 1 c.c. of raw milk, and the lowest count was 6,467,810 bact. per 1 c.c. of boiled milk.

The lactose agar plates method gave a count of 85,900,000 bact. per 1 c.c. of raw milk as a maximum, and a count of 3,000,000 bact. in boiled milk as a minimum. The highest and lowest counts obtained by each method in raw, mixed and boiled milk samples are tabulated in the following table:

	Raw.	Mixed.	Boiled.
Out of 200 milk samples.	76 samples (38%).	80 samples (40%).	44 samples (22%).
Skar's: Highest count	332,108,006	196,302,929	154,395,562
Lowest count	7,625,250	7,625,250	6,467,810
Lactose agar plate: Highest count ...	85,900,000	82,000,000	38,000,000
Lowest count ...	8,500,000	3,400,000	3,000,000

The highest count has been generally in August and September, and the lowest in January and February.

The B. coli titre was determined by means of Kessler's and Swenarton's medium. The different B. coli titres we obtained from the 200 milk samples were as follows:

B. coli per 1 c.c.	Number of samples.	Percentage.
1,000,000	81	40.5%
100,000	89	44.5%
10,000	26	13.0%
1,000	4	2.0%

Knowing that 1 gram of fresh cow's manure contains about 50,000,000 bacteria, one may imagine by what unsanitary methods the market milk of Cairo

is produced and handled. The tremendously high bacterial counts may be partly due to the unhygienic conditions of production and partly due to the lack of milk cooling, so that the atmospheric temperature under which milk is usually kept from the time of its production until it reaches the consumer favours the multiplication of milk organisms to such great numbers.

Part II. (a) Mycobacterium tuberculosis: Out of the 200 samples, two (Nos. 107, 159) were found to contain tubercle bacilli, i.e., 1 per cent. In sample No. 107 the tubercle bacilli could only be detected and isolated from the pus of the local abscess in the injected guinea-pig which died after 21 days. The pus was then treated and spread on Wahby's egg-medium, on which typical growth could be seen clearly after two weeks; films were also made and examined microscopically. Subcultures were then made on four different media: Wahby's, Dorset's egg-gentian-violet, glycerine agar, and glycerine coagulated ox serum. Growth was already visible on Wahby's after one week, whereas on the other media the colonies could not be seen before three to four weeks. So Wahby's medium proved to be more favourable, as it gave quicker and more luxuriant growth than Dorset's, glycerine agar, and glycerine coagulated ox serum.

The typing of the isolated bacilli was made by injecting an emulsion from the culture subcutaneously into a mature rabbit, which died after 18 days with tuberculous lesions in the intestines, liver, lungs, inguinal lymph glands and a local abscess with lacerations of the skin at the site of inoculation.

Films made from these lesions proved the presence of tubercle bacilli; thus they were typed as bovine and kept on egg and glycerine agar media. In this sample the cultural and microscopical examination failed to give positive results.

On the other hand, in sample No. 159 the organisms were isolated from both the inoculated animal and the culture. The injected guinea-pig died 16 days after inoculation, and the bacilli were easily isolated from the lesions in the previously-described manner. On the culture the tubercle bacilli colonies made their appearance after 23 days' incubation. On typing them they proved to be of the bovine type; they killed a mature rabbit in 21 days. The microscopic examination of this milk sample gave negative results; neither acid fast bacilli nor cell-groups could be detected.

Thus it may be concluded that the animal inoculation still stands as the best method for detecting and isolating tubercle bacilli from milk. The cultural method gave only 50 per cent. of the positive results compared with the animal inoculation method. In none of the positive cases the cell-grouping advocated by Torrance, Matthews and Maitland was detected, so it seems that this method of recognising tuberculous milk is not applicable to market milk, although it may be valuable in examination of individual samples from tuberculous udders. This conforms with the findings of Kendall, Gwilyn and Davis.

(b) Brucella abortus: As milk of infected animals contains Brucella agglutinins, many authors suggested various titres for the positive agglutination test of milk serum of infected animals varying from 1:10 to 1:80 or over. Gohar considered a titre of 1:80 or over as positive, as Br. abortus seems to share an antigenic factor with Salm. paratyphi B, B. dys. Flexner, as well as some strains of B. coli. On the other hand, Klimmer stated in his monograph on Br. abortus that one might find a whey titre as low as 1:25, or lower in the milk of 13 per cent. of animals disseminating Br. abortus and a whey titre of 1:50 or lower in the

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milk serum of 29 per cent. of such animals. Pröscholdt and Karsten found that 25 per cent. of the animals excreting Br. abortus in their milk gave a whey titre as low as 1 :10, and 64 per cent. of such animals gave a milk whey titre of 1 :40.

However, these titres are usually applied on individual milk samples, but no definite whey titre can be possibly suggested for the market milk on account of the wide fluctuations of dilutions to which the infected milk is subjected on bulking, and due to the probable destruction of agglutinins of the whey by boiling or heating the milk to a high temperature, as it is customary in this country.

The results obtained in this work by the agglutination test of milk serum are shown in the following table.

Number of positive samples.	Titres. 1 :10	1 :20	1 :40
26 samples	+ +		
7 (Nos. 62, 85, 101, 115, 127, 183, 199) ...	+ +	+ +	
3 (Nos. 25, 150, 173)	+ +	+ +	+ +
Total number of positive samples	36	10	3
% of positives in relation to total number of 200 samples	18%	5%	1.5%

If we consider the 1 :40 dilution a positive titre, then the percentage of the incidence of Br. abortus in market milk of Cairo is 1.5 per cent., and if we accept the 1 :20 dilution a positive titre, then we may have an incidence percentage of 5 per cent. As previously stated, we cannot ourselves set a definite titre for the market milk infected with Br. abortus.

However, the abortus organisms were actually isolated from sample No. 127, which gave an agglutination reaction at 1 :20 whey dilution. In this sample the organisms were isolated from the enlarged spleen (with no visible lesions) and liver of the inoculated guinea-pig which died 23 days after inoculation by culturing on gentian-violet-liver-agar and incubating under a 10 per cent. CO_2 atmosphere. The isolated organisms were typed as Br. abortus by the two previously-mentioned methods of thionin-agar and agglutination by the serum of two previously-mentioned methods of thionin-agar and agglutination by the serum of immunised rabbit. They could not grow on thionin-agar, and they gave a positive agglutination test at a titre of 1 :800. So we may state that we have isolated Br. abortus from market milk of Cairo in 0.5 per cent. of the examined samples.

In addition, out of the 200 guinea-pigs inoculated, 85 remained alive; they were killed 7 weeks after inoculation and blood serum could be collected only from these guinea-pigs for the agglutination test. Only 7 guinea-pigs gave a positive agglutination test at a titre of 1 :10 of their blood serum; the other 78 sera gave negative results. The remaining 115 guinea-pigs died spontaneously from a few days to a few weeks after inoculation, and obviously we could not collect their sera.

The seven positive guinea-pigs were inoculated with samples Nos. 21, 41, 50, 85, 153, 101, and 183. The whey of these samples gave positive agglutination at titres varying from 1 :10 to 1 :20, except No. 41, which was absolutely negative.

According to Klimmer, the titre of the normal guinea-pig serum always lies

under 1:5, and the appearance of agglutination titre of 1:5 or over in the serum of the inoculated guinea-pigs should be considered positive as to abortus infection. If we accept the 1:10 titres obtained as positive, then we would have 8.2 per cent. in proportion to the 85 examined sera, or 3.5 per cent. in proportion to the total number of 200, as incidence percentage of Br. abortus in Cairo market milk.

Thus the incidence of Br. abortus in the examined milk samples can be safely estimated at 3.5 per cent., according to the animal inoculation method.

(c) The enteric fever group: It was possible to isolate Salmonella paratyphi B from one sample (No. 73) out of the 200 samples, i.e., 0.5 per cent.

Pale, nearly colourless colonies were found on McConkey's medium after 24 hours' incubation at 37° C. Films stained with Gram's proved to contain Gram-negative, non-sporulating rods. Separate colonies were picked and transferred to sugar tubes; they gave the following reactions:

In 1 per cent. lactose broth, no change, and in 1 per cent. glucose broth and 1 per cent. mannite acid and gas were produced. Thus the organisms were considered paratyphoid bacteria. For further indentification, litmus milk tubes were inoculated. Slight acidity, which turned later on to alkalinity, was produced, the organisms were then suspected to be paratyphoid B. Emulsion of the organisms was made to run agglutination test against two sera, one paratyphoid B and the other paratyphoid A. The paratyphoid B serum agglutinated the organisms at a dilution of 1:480, while the paratyphoid A serum did not agglutinate them at all. So, the isolated organisms were typed Salmonella paratyphi B.

Thus the incidence percentage of typhoid organisms was nil, while that of paratyphoid B was 0.5 per cent.

This result should not be considered conclusive on account of the difficulty encountered in isolation of these organisms from such milk with extraordinarily high bacterial count as the examined milk.

Summary

(1) Two hundred samples of Cairo market milk were examined; among them there were 76 raw, 44 boiled, and 80 mixed and boiled samples.

(2) The highest count obtained by Skar's was 332,108,006 bacteria per 1 c.c. of raw milk, and the lowest was 6,467,810 bacteria per 1 c.c. of boiled milk. Skar's gave better results than Breed's method in the routine work.

(3) The lactose agar plate method gave a count of 85,900,000 colonies per 1 c.c. of raw milk as a maximum, and a count of 3,000,000 colonies per 1 c.c. of boiled milk as a minimum. The lactose agar proved to be equal to skimmed-milk agar, but more transparent.

(4) The bacterial counts were higher in summer than in winter months.

(5) The highest B. coli titre obtained was 1:1,000,000, and the lowest was 1:1,000. Kessler's and Swenarton's medium was superior to lactose broth, and equal to McConkey's bile salt lactose broth.

(6) The cell-grouping after Torrance and Matthews, as well as the microscopic detection of tubercle bacteria, did not prove of much value in the routine examination of market milk.

(7) The tubercle bacilli were isolated from two samples of milk by animal

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inoculation (1 per cent.), whereas by culture they could be only detected in one sample (0.5 per cent.). The isolated organisms were typed bovine by rabbit inoculation.

(8) Wahby's egg medium proved superior to Dorset's egg-gentian-violet, glycerine agar and glycerine ox serum media in cultivating the tubercle bacilli.

(9) Out of the 200 samples of milk sera, 3 agglutinated Br. abortus at a titre of 1 :40 (1.5 per cent); 10 agglutinated the organism at a titre of 1 :20 (5 per cent.); and 36 sera at 1 :10 (18 per cent.). It was only possible to isolate the organism from one sample (0.5 per cent.) by animal inoculation (enlarged spleen). This sample gave an agglutination whey titre of 1 :20.

Only 85 of the inoculated guinea-pigs could be serologically examined; out of them, 7 guinea-pigs gave 1 :10 blood serum titre, i.e., 3.5 per cent. in proportion to the total guinea-pigs number. There was no correlation between the whey titres and the blood serum titres of these animals.

(10) Salmonella paratyphi B was isolated from one milk sample out of 200 examined samples by the brilliant green enrichment method (0.5 per cent.).

(11) All samples, from which pathogenic organisms were actually isolated or their presence was highly probable (high agglutination titres), were either of raw or of mixed (raw and boiled) milk.

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ABSTRACT

HORMONAL STIMULATION OF ESTRUS AND OVULATION IN SHEEP AND GOATS*

(A Review)

By RALPH W. PHILIPS, Ph.D., RICHARD M. FRAPS, Ph.D. and ARCHIE H. FRANK, D.V.M., M.A., *Beltsville, Maryland.*

THE possibility of stimulating estrus and ovulation in anestrous does and ewes with hormones has received considerable attention in recent years. Results have been variable, and techniques that give dependable and satisfactory results in practice have not yet been developed. The object of this paper is to present a review of the work conducted to date, so that field workers and others interested in the application of these results in practice may be acquainted with the present status of the problem.

The results of the many attempts described in the literature to induce estrus and ovulation in anestrous sheep and goats by the use of gonadotropic hormones are summarised by the present authors as follows:—

(1) Follicular growth and ovulation can be induced in a high percentage of animals injected with gonadotropins.

(2) Induction of estrus in conjunction with ovulation, as a result of treatments with gonadotropins, has been quite erratic, and no satisfactory explanation of the variable results has been found.

(3) Fertility, as measured by percentage of conceptions, is generally lower in animals in which estrus has been induced or in those force-mated after induction of ovulation, than in animals bred during natural estrus. However, satisfactory fertility has been reported in some cases.

Estrogens, used either alone or with gonadotropins, have given variable results. Results of trials with gonadotropins and/or estrogens during the natural breeding season have also been quite variable. In some cases, apparent increases in fertility, as judged by percentage of conceptions or lambing percentages, have resulted. In other cases, no increase or an actual lowering of fertility has resulted.

The variable results are difficult to explain. The identity of hormone preparations can never be assured in the absence of chemical purity, and no protein gonadotropin is now marketed in this form. It does not appear probable that this accounts in any large measure for the inconstancy of estrus response in ewes and does, owing to the relatively uniform response of laboratory animals to these same gonadotropins.

Constitutional and environmental factors may play a considerable part in determining the response to specific hormone treatments. These factors, if they do play an important part, are easily overcome in the induction of follicular growth and ovulation. The extent to which they influence expression of estrus is not clear, and cannot be fully understood until the mechanism of induction of estrus is determined in ewes and does.

* *American Journal of Veterinary Research*, July 1945.

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From work with laboratory animals, and the results with sheep and goats, it seems certain that time sequences in hormonal action are involved critically in the expression of normal and induced estrus. There is obvious need for further work to determine (1) the endocrine physiology of normal and experimentally induced estrus in sheep and goats, and (2) the specific doses and time sequences of hormones that will induce estrus and ovulation in anestrous ewes and does.

Abstracts of Current Literature

FROZEN MILK FOR HOSPITAL SHIPS*

THE milk furnished on hospital ships is pasteurised, homogenised, and kept in a frozen condition until thawed for use. To save space, attempt was made to use a frozen concentrated milk which was to be reconstituted on board ship by the addition of water after thawing. Such milk was not satisfactory, as it is difficult to reconstitute it in a sanitary manner and it frequently develops an abnormal flavour when stored for short periods. Frozen milk in 5-gallon cans was also used to some extent, but was discontinued because of health hazards encountered in the use of bulk milk.

Specifications for pasteurised frozen milk have been approved and are about ready for distribution. They permit the use of only fresh milk which meets the specification requirements for Type II pasteurised milk, and require that the milk shall be properly homogenised, packaged in rectangular paper containers having a capacity of one quart, and frozen solid within not more than twenty hours. The milk will be purchased by the quartermaster and will be produced, processed, and handled under the inspection of the Veterinary Corps. The procurement of milk under these specifications will ensure that safe milk of good quality is furnished to hospital ships.

Packaging the milk in paper quart containers not only permits the milk to be thawed as needed, but thawed without exposure to contamination. Some separation of fat and casein takes place in milk when it remains frozen for a prolonged period. Reports, however, show that milk meeting these specifications has been very favourably received by patients on hospital ships after it had been stored for three months.

* The Bulletin of the U.S. Army Medical Department.

TOPOGRAPHY OF TEAT MUCOUS MEMBRANE†

POST-MORTEM examination shows that some teats have a smooth mucous membrane; some are slightly pocketed and some are markedly pocketed. It seems logical to suppose that this pocket or pouch formation might tend to hold back milk at the time of milking, and in this way serve as a reservoir for mastitis infections. Observations have been made and recorded to provide more information on this phase of the mastitis problem.

Examination of the teats from 11 Guernsey and 10 Holstein-Friesian cows showed that in any cow all four teats tended to classify in the same group. They

† *Cornell Veterinarian*, January, 1945.

also showed that there was a higher percentage of smooth mucous membranes among the Guernsey cows examined than among the Holstein-Friesians, and that there was no recognizable relationship between the degree of pocketing and the incidence of mastitis infections. The presence of mastitis-producing bacteria in milk samples, examined during life, did not serve as a reliable guide for determining the topography of the teat mucous membrane.

CANCER IN COW'S UDDERS IS RARE*

SCIENTISTS are baffled by the fact that examination of the udders of 13,000,000 cows has failed to reveal the presence of cancer in this gland of cattle. This condition is hard to understand because cancer is common in the corresponding glands of human beings and of other animals, and the absence of cancer in cows' udders is still more surprising because that organ is unusually large, highly developed functionally and is exposed to irritation and bruises.

Last year, post-mortem examinations by the federal meat inspection service disclosed that 32,709 cattle were affected with some kind of tumor, and laboratory studies showed that many were malignant, but no cases of cancer of the udder were found.

* U.S.D.A. Research Administration, May 29, 1945.

BLOOD CHANGES IN COWS AT PARTURITION†

THE analysis of cow's blood for calcium, phosphorus, magnesium and vitamin B, four weeks before calving, within 12 hours before and after calving, and one week after calving was made at the Ohio Agricultural Experiment Station on 80 Jerseys, 35 Holstein-Fresians, 21 Ayrshires and 15 Guernseys to determine the blood changes that take place at freshening time in both normal and milk fever cows. In both the healthy and the sick cows there was a slight lowering of calcium and phosphorus just after freshening. During the attack of milk fever, the calcium level dropped 40 per cent. and the phosphorus level 30 per cent. below normal, while magnesium increased 75 per cent. The high increase in magnesium may account for the anesthetic state of milk fever in lieu of the tetany to be expected in hypocalcemia. A significant finding was that the vitamin D level of cows that developed milk fever was considerably lower than in normal cows four weeks before calving, but giving a million units of vitamin D daily, during the pre-freshening four-week period, did not prevent the disease in all cases.

† *Journal of Animal Science,* November, 1944.

SALT FOR LIVESTOCK‡

SALT is the foremost mineral deficiency of the livestock ration. It frequently accounts for depraved appetites when insufficient. According to studies made at the University of Wisconsin and the agricultural experiment stations of the Dominion of Canada and Iowa, salt-starved sheep, pigs and cows show low efficiency in utilising feed. By actual tests made by Bohstedt, of Wisconsin, one pound of salt saved many pounds of grain. In order that animals may satisfy their needs, salt should be fed on a free-choice basis, especially when oil meals

‡ *Poultry Science,* April, 1944.

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and mill feeds are used to supplement the home-grown feeds. The same findings apply to poultry. The depraved appetite manifested by cannibalism among growing chicks is cured by feeding salt. Sodium chloride of various feed runs from 0.10 per cent. for corn to 0.40 per cent. for tankage, while the percentage in animal tissues and secretions varies from 0.20 for milk to 0.80 for saliva. Beef and eggs contain about 0.35 per cent., milk 0.30, etc., through various levels. The sodium chloride levels of livestock feeds being lower than that of the body tissues and fluids, the deficiency must be supplied in the feed.

HUMAN AND ANIMAL BRUCELLOSIS*

THE problem of brucellosis in cattle, swine and man, which is far from being brought under control, was discussed at length at the annual meeting of the American Veterinary Medical Association by foremost figures in the field of livestock sanitation. Although the disease in man was little more than a curiosity 25 years ago, it was declared to be more prevalent now than typhoid fever in many states, and not anywhere appreciably reduced by control measures either in man or animals, owing to the insidious nature of the infection. Being the more vulnerable, the farm population was said to be more and more alarmed over the heavy losses of cattle and the increasing danger of contracting the disease from them.

In swine, exact statistics on its prevalence are lacking, but in general, particularly in the more congested swine centres, the losses suffered are known to be great, and the danger to man and cattle admitted. Swine-to-cow-to-man epidemics occur, and vaccination has had no beneficial action in swine.

In cattle, resort to the test-and-slaughter method was pronounced meritorious, provided it is faithfully carried out, and found to be economically justified. In the case of so-called "storms of abortion" it was, however, pronounced unscientific and economically unsound, because repeated testing and slaughtering finally takes all of the herd, since new disease-free additions eventually become infected. Summed up, the discussion revealed that brucellosis is a major threat to the health of cattle, swine and human beings, and that an all-out programme for its control must be centred upon farm animals, based on (1) the strict sanitary measures required to master infections, (2) test and slaughter where economically feasible, (3) calfhood vaccination (with strain 19 vaccine) to build up immune herds, and (4) vaccination of the whole herd in the case of "storms of abortion" and where the percentage of infected animals is high.

* *Journal of the American Veterinary Medical Association*, March, 1945.

OCCUPATIONAL ALLERGY AMONG VETERINARIANS†

THAT the human race is becoming allergic to many substances more generally than in former times is supported by the large number of veterinarians who suffer from vesico-pustular dermatitis of the hands and arms, which is characterised by exacerbation and remissions corresponding to their exposure (contact) with certain foreign substances, such as body tissues and fluids in animals, vaccines, bacterins, antitoxins, fungi, and chemical agents, including harsh soap, employed in the pursuit of their daily work. The trouble is chronic and difficult to overcome. It is manifested by rash, pruritis, multiple vesication and pustulation of the hands and arms, seldom of any other part of the body. Exposure

† *Jen-Sal Journal*, January-February, 1945.

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to the vaginal discharges of cows infected with brucellosis is one of the more common causes. The dermatitis is so severe and persistent that not a few veterinarians have had to discontinue practising at frequent intervals or entirely. A survey revealed that approximately 20 per cent. of all American veterinary practitioners suffer to some extent from allergic dermatitis of the hands and/or arms. The type caused by vaginal discharges of cows can be prevented and favourably modified by the use of a high titer Brucella antiserum (Huddleson). Considerable benefit is also derived by taking daily doses of sodium bicarbonate and calcium lactate, but the main remedy is to avoid hand washing that tends to damage or destroy the cutaneous epithelium of the hands.

Review

Biological Actions of Sex Hormones. By Harold Burrows, C.B.E., Ph.D., F.R.C.S. Cambridge University Press: 1945. Price 42s. net.

THERE are few subjects of biological application which have made such rapid progress in recent years as the study of sexual and reproductive phenomena. The main advances in this field have been made in the discovery and isolation of the "sex hormones," which are responsible for the control and regulation of reproductive processes in mammals. By far the greater part of present knowledge concerning the functions of these hormones has been obtained from experimentation with laboratory rodents, and much of the numerous data is scattered in many publications in various languages which are not easily available except to the endocrine specialist. It is stated in the preface to this book that the author's aim was to present "a co-ordinated summary of experimental inquiries in this field," and it was, therefore, with no little anticipation that the book would meet the needs of the more general reader in this connection that we turned to its subject matter. On first glancing through the book, one cannot help being impressed by the immense array of publications which the author has consulted, but after careful reading it must be confessed that our high hopes were not completely fulfilled. This is not due to any shortcoming in the way in which the author approaches his subject matter, but rather to the inherent difficulty of transforming a compilation of references into a readable narrative. A more serious point is that the author has not been sufficiently critical in his selection of publications for review, many of those summarised being but of transient interest and adding nothing materially to our understanding of the problem as a whole.

The book is divided into six parts, each covering the research literature dealing with one of the sex hormones. Part I is concerned with the nature and functions of the gonadotrophic hormones of the anterior pituitary, the factors which influence the gonadotrophic activity of the pituitary, and the factors which influence the reaction of the gonads to gonadotrophins. Part II introduces the subject of gonadal hormones, which are then exhaustively covered in subsequent chapters, under the headings "Androgens," "Œstrogens," and "Progestins" respectively. Part III comprises a summary of the knowledge regarding the sex hormones of the adrenal cortex.

There are several inaccuracies which cannot be overlooked in a review in a

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veterinary journal. Thus it is somewhat surprising to read in a book, the manuscript of which appears to have been completed in 1944, that the gonadotrophic hormone of pregnant mares' serum is thought to take place in the pituitary, and not in the placenta (see p. 6, p. 12, p. 80, and p. 457). On p. 18, the sheep and the pig are included among the animals which usually do not ovulate in the absence of coital stimulus. The author's authority for this statement is given as Heape (1905), but surely there have been more recent, and more accurate, observations in this connection since Heape's time. On p. 20 the statement is attributed to Parkes and Hammond (1940) that ovulation can be induced in sheep during œstrus (*sic*) by injections of equine pituitary; this looks like a misprint of "anœstrous." On p. 44, Heape (1901) is again cited for the observation that monœstrus animals have but *one œstral season in the year*. The statement is made on p. 30 that "both in seasonal breeders and in those animals which retain reproduction capacity throughout the year, the gonads after puberty can be aroused into action at any time by the administration of gonadotrophin"; a study of veterinary literature would, however, have revealed that this is very far from being the case.

There are also several statements of a more general nature which are not in accord with orthodox opinion in endocrine matters. For example, on p. 19 there occurs the surprising statement that "the pituitary has little direct control of the production of ova, the formation of which from the germinal epithelium is a response to œstrogen," and on pp. 287 and 282 the observations of Allen (1923) and Bullough (1942) on ovogenesis are cited in support of the view that "it seems proved that the development of new egg cells is dependent on œstrogen."

Despite its shortcomings, some of which we have enumerated above, this book should prove useful as a reference work to the literature dealing with the experimental studies in sex endocrinology, provided the user has sufficient knowledge of the subject to enable him to evaluate the material quoted.

N.J.S.

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